

Polyol metabolism in homopterans at high temperatures: accumulation of mannitol in aphids (Aphididae: Homoptera) and sorbitol in whiteflies (Aleyrodidae: Homoptera)¹

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Received 19 December 1997; received in revised form 10 March 1998; accepted 24 March 1998

Abstract

Examination of four species of aphid and four of whitefly showed that mannitol was present in each species of aphid, while sorbitol was present in the whitefly species. In the cotton aphid, *Aphis gossypii* Glover, the total body content of mannitol was considerably higher at noon than during the early morning. A similar increase in the sorbitol content of the silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring, was also demonstrated. In both species, polyol synthesis is stimulated by elevated temperatures. Enzyme assays were used to show that fructose is the substrate for mannitol synthesis in *A. gossypii*. The enzyme catalyzing this reaction, an NADP(H)-dependent ketose reductase/mannitol dehydrogenase, is analogous to the NADP(H)-dependent ketose reductase/sorbitol dehydrogenase that produces sorbitol in whiteflies. Western blot analysis verified that *A. gossypii* does not contain a protein that cross-reacts with antibodies against *B. argentifolii* NADP(H)-dependent ketose reductase/sorbitol dehydrogenase, whereas the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood, does. Analysis of sugars in honeydew from aphids and whiteflies showed that the sugars in the excrement from these insects are very different from the sugars present in their bodies. Only small amounts of mannitol and sorbitol are excreted in the honeydew from these insects. Sorbitol accumulation provides a mechanism for thermo- and osmoprotection in whiteflies. Mannitol appears to function in a similar capacity in aphids. © 1998 Elsevier Science Inc. All rights reserved.

Keywords: Aphids; Whiteflies; Honeydew; Ketose reductase; Mannitol dehydrogenase; Sorbitol dehydrogenase; Thermoprotection

1. Introduction

Whiteflies and aphids are homopteran insects that feed upon plant phloem sap. Many aphid and whitefly species live in hot arid regions where they cause serious agricultural problems. We showed previously that the polyhydric alcohol (polyol) sorbitol accumulates in high

concentration in the hemolymph of the silverleaf whitefly (*Bemisia argentifolii*, Bellows and Perring), especially under warm (> 35°C) temperature regimes [38]. The sorbitol content of the bodies of whiteflies fluctuated diurnally and was proportional to both the osmotic strength of their diet and to environmental temperature.

In *B. argentifolii* sorbitol was synthesized from the sucrose present in its diet of plant phloem sap. Ingested sucrose is converted to fructose and glucose in the insect by sucrase (i.e. α -D-glucopyranosidase) [25] and the resulting fructose serves as the substrate for sorbitol biosynthesis [26,38]. In most organisms, the conversion of fructose to sorbitol is catalyzed by the catabolic

Abbreviations: KR, ketose reductase; PVDF, polyvinylene difluoride; SDH, sorbitol dehydrogenase; SDS-PAGE, sodium dodecyl-sulfate polyacrylamide electrophoresis.

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enzyme sorbitol dehydrogenase (SDH EC 1.1.1.14). In the whitefly, this reaction is catalyzed by an anabolic ketose reductase (KR) known as NADPH-dependent KR/SDH (NADPH-KR/SDH). A novel feature of the whitefly enzyme is its use of NADP(H) as a coenzyme [26]. All other SDHs require NAD(H) [19].

Mannitol and sorbitol are both straight chain hexitols. Mannitol, the most abundant acyclic polyol in nature [4], is not widespread in the animal kingdom, having been reported in only a few insects [14,29,30]. In fungi and microorganisms, mannitol synthesis is catalyzed by mannitol dehydrogenases which use either NADPH or NADH [7,35]. Unlike mannitol, sorbitol commonly occurs in animals [8,22,32], including humans [18]. Both of these polyols are compatible organic osmolytes that protect organisms from cold [14,20,22,30–32] and osmotic stress [2,13,21,27]. Polyols are also known to protect proteins from denaturation at high temperature [10]. Recent studies with *B. argentifolii* suggest that sorbitol accumulation improves the survival of this insect at high temperature [38].

In the present study, we analyzed the polyol content of several whitefly and aphid species. We wished to determine: (1) if all whiteflies accumulated sorbitol, or whether this phenomenon was limited to *B. argentifolii* and (2) if aphids also accumulated polyols at elevated temperatures and, if so, the biochemical mechanism for polyol accumulation in aphids.

2. Materials and methods

2.1. Insect material

Silverleaf whiteflies, *B. argentifolii* Bellows and Perring, the cotton aphid, *Aphis gossypii* Glover, and the green peach aphid, *Myzus persicae* Sulzer, were reared in greenhouses on upland cotton plants, *Gossypium hirsutum* L. var. Coker 100A glandless, as described previously [25]. The greenhouse whitefly, *Trialeurodes vaporariorum*, was reared on tomato plants, *Lycopersicon esculentum* Mill., in a greenhouse in Riverside, CA. The banded wing whitefly, *T. abutilonea* Halde- man, was collected in a cotton field in Phoenix, AZ. The shallot aphid, *M. ascalonicus* Doncaster, was collected from collard plants, *Brassica oleracea* var. *acephala* DC, growing in a field in Phoenix and the blackmargined aphid, *Monellia caryella* Fitch, was collected from pecan trees, *Carya pecan* Engler & Graebn., in a commercial orchard in Maricopa, AZ. The acacia whitefly, *Tetraleurodes acaciae* Quaintance, was collected from purple orchid trees, *Bauhinia variegata* L., in Phoenix, AZ. In all collections, the insects were collected both in the early morning (ca. 07:00) and shortly after noon (ca. 13:00) during the months of July and August. For insects collected from both the field

and greenhouse, air temperatures during the morning and afternoon collections were 26–28 and 38–42°C, respectively. In some experiments, insects were fed on cotton plants at various temperatures as described earlier [25]. The temperature of the plants was regulated by placing them inside thermostated chambers [38].

2.2. Sugar and polyol analysis

Immediately following collection, insects were placed into either ice-cold 80% ethanol or liquid nitrogen for transport to the laboratory. In the laboratory, insects were extracted several times in hot (80°C) 80% ethanol and aliquots of the pooled extracts were treated with activated charcoal to remove materials which interfered with subsequent chromatography and enzymatic analyses [15]. The charcoal-treated extracts were filtered through a 0.2 µm filter and evaporated under N₂. The resulting residues were resuspended in deionized water for injection onto two Dionex PA1 columns in series. Carbohydrates and polyols were eluted from these columns with 0.2 M NaOH in which a sigmoidal gradient of 0–0.5 M sodium acetate was introduced at sample injection [17]. The sugars and polyols that eluted from these columns were detected by pulsed amperometry.

The identity of mannitol and sorbitol in these extracts were confirmed by several methods including (1) their retention times on Dionex PA1 and BioRad Aminex HPX-42C HPLC columns, (2) their resistance to 0.5 N NaOH at 100°C, and (3) their reaction with enzymes specific for these polyols ([38] and see text). For enzymatic confirmation of mannitol, an aliquot of the alcoholic extract from the insects was evaporated and the resulting residue suspended in buffer and incubated with mannitol dehydrogenase. The complete reaction mixture contained 0.1 M Tricine/KOH, pH 8.5, 20 mM NAD⁺ and 1 IU of mannitol dehydrogenase from *Leuconostoc mesenteroides* (EC 1.1.1.67; Sigma M9532). The reaction mixtures were incubated at 32°C for 4 h after which time the reaction was terminated by placing the tubes in a boiling water bath for 2 min. A tube containing the identical reaction mixture lacking NAD⁺ and one in which the mannitol dehydrogenase was inactivated by heating prior to the reaction were used as controls. The product of the mannitol dehydrogenase reaction was detected by HPLC as described above. Similar assays using SDH were conducted to confirm the identity of sorbitol [38].

2.3. Honeydew collection and analysis

Honeydew excreted by *B. argentifolii* and *A. gossypii* was collected by placing aluminum foil under insects feeding on cotton leaves in a greenhouse. After honeydew deposits accumulated for 24 h, they were re-

moved from the foil with hot deionized water and concentrated by lyophilization. Sugars in this honeydew were analyzed by anion exchange HPLC employing the same procedures employed for insect polyol analysis. The identity of sugars in the honeydew samples were determined by: (1) comparison of their retention on several different HPLC columns with that of known sugar standards, (2) treatment of the extracts with enzymes specific for various sugars, and (3) NMR and mass spectroscopy analysis of sugars isolated from HPLC eluates [17,36,37].

2.4. Enzyme extraction and assay

Extracts of adult whiteflies and aphids were prepared by homogenizing the insects at 4°C in 50 mM Hepes/KOH, pH 7.9. The extracts were centrifuged for 10 min at 10000 g and the supernatant used for enzyme assays. Ketose reductase activity in the extracts was measured at 30°C as described previously [38]. Protein was determined by the method of Bradford [6].

2.5. Analysis of reaction products

Products of the ketose reduction reaction were determined by incubating soluble whitefly and aphid extracts with 10 mM NADPH or NADH and 425 mM fructose in 50 mM potassium phosphate, pH 7, for 3 h at 30°C. Reactions were terminated by placing the tubes in a boiling water bath for 2 min. After boiling, the reaction mixtures were centrifuged for 1 min to remove precipitated protein and the supernatant diluted 30-fold for polyol and sugar analysis by anion-exchange HPLC [17]. Whitefly and aphid extracts that were heat-denatured by heating for 2 min at 100°C prior to incubation were used as controls.

2.6. Western blot analysis

Western blot analysis of whitefly and aphid extracts was conducted as described previously [26]. Polypeptides in the extracts were separated by SDS-PAGE, transferred to Immobilon-P polyvinylene difluoride (PVDF) membrane and the membrane probed with antibodies directed against whitefly NADPH-KR/SDH [26].

3. Results

Chromatograms of body extracts of *A. gossypii* contained three major peaks, corresponding to trehalose, glucose and an unknown compound that eluted at 4.2 min (Fig. 1). The retention time of this unknown peak was identical to that of a mannitol standard. Glucose and trehalose levels in *A. gossypii* at 07:00 and at noon

were similar, whereas the putative mannitol peak was markedly higher in extracts of insects collected at noon.

Analysis of *A. gossypii* extracts after reaction with mannitol dehydrogenase showed that this enzyme treatment caused the putative mannitol peak to decrease in size compared with the control reactions which lacked NAD⁺, the cofactor utilized by mannitol dehydrogenase (compare Fig. 2A and B). A concomitant increase in the size of the fructose peak (the product of the reaction catalyzed by mannitol dehydrogenase) was noted following enzyme treatment of the presumed mannitol peak (Fig. 2B). The sizes of the putative mannitol and fructose peaks in these chromatograms did not change from that of the controls lacking NAD⁺ in a second control experiment in which *A. gossypii* extracts were incubated with heat-denatured mannitol dehydrogenase (compare Fig. 2A and C). Heating of cotton aphid extracts in 0.5 N NaOH at 100°C did not affect the size of the mannitol peak (data not shown), which would be expected for acyclic polyhydric alcohols such as mannitol, which are resistant to this treatment.

As observed previously [33], sorbitol levels in *B. argentifolii* increased markedly from early morning to noon (Fig. 1). The sorbitol content of these insects was 10-fold higher in the early afternoon than in the early morning or late afternoon. The other major peaks in the chromatograms of whitefly body extracts, corre-

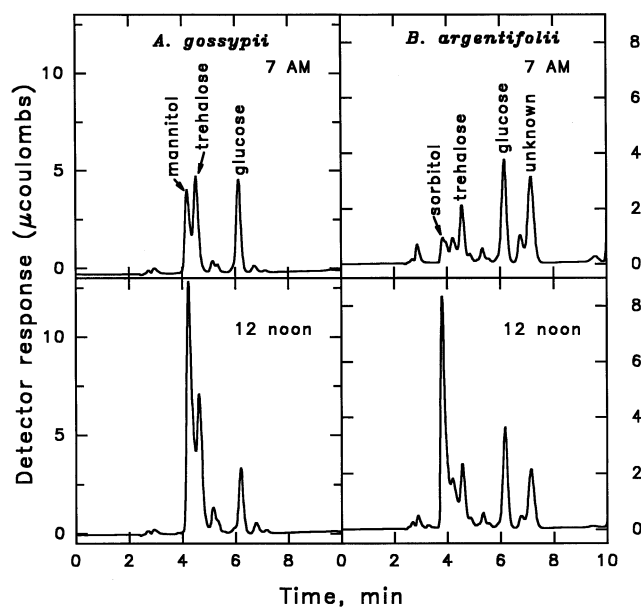


Fig. 1. Polyols and sugars in extracts of *A. gossypii* and *B. argentifolii* at two times during the day. Anion-exchange HPLC profiles of the 80% ethanol-soluble extracts of aphids and whiteflies collected at 07:00 (top panels) and 12:00 (bottom panels). Panels represent HPLC analysis of extracts of either 100 silverleaf whiteflies or 25 cotton aphids. The amount of polyol per insect was calculated from the area of the appropriate peak in these chromatograms compared with the area of peaks of injected standards.

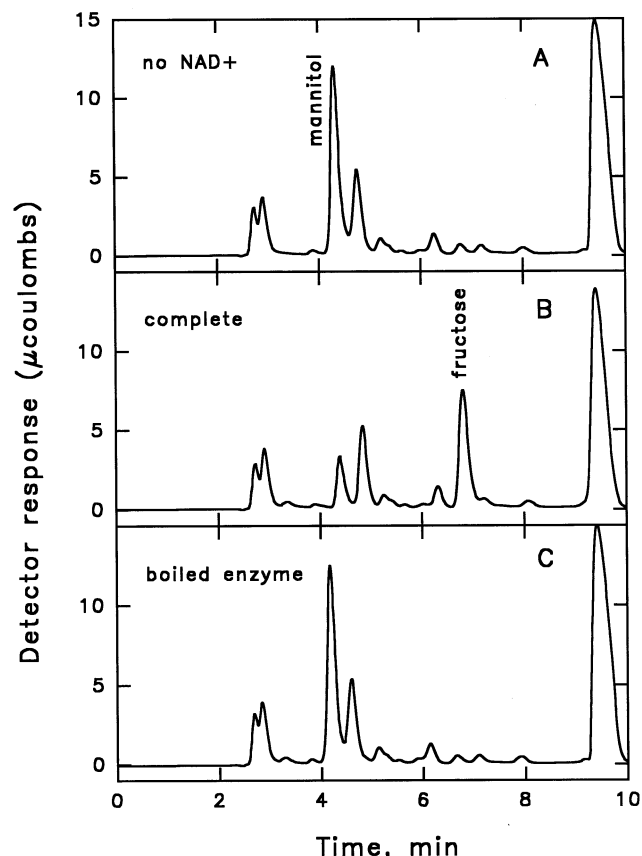


Fig. 2. Identification of mannitol in *A. gossypii*. Anion-exchange HPLC profiles of the 80% ethanol-soluble extract of aphids after incubation with mannitol dehydrogenase in the absence (panel A) or in the presence of NAD⁺ (panel B) or with NAD⁺ plus a mannitol dehydrogenase solution that had been placed in a boiling water bath prior to incubation with the aphid extract (panel C).

sponding to trehalose, glucose and an unidentified compound, were of similar size in chromatograms of extracts from insects collected in the morning and at noon ([38], Fig. 1).

A second HPLC column (BioRad Aminex HPX-42C), which provided excellent separation of mannitol from sorbitol, fructose, glucose and trehalose, was used to analyze aphid and whitefly extracts. Retention times for these carbohydrates on this second column were 21.5, 26, 17.3, 14 and 12 min, respectively (not shown). When insect body extracts were chromatographed on this column, a peak corresponding to mannitol was detected in chromatograms of *A. gossypii* extracts and a peak corresponding to sorbitol was detected in chromatograms of *B. argentifolii*.

Sorbitol levels in *B. argentifolii* were at least 5-fold higher at 42°C than at 28°C (Fig. 3). Mannitol was likewise found to be substantially higher in *A. gossypii* bodies collected at 38°C than those collected at 23°C (Fig. 3). Little change was found in levels of trehalose and glucose in extracts from either species of insects collected at low or high temperatures.

To determine the prevalence of mannitol accumulation among aphid species, we analyzed the body contents of three other aphid species: *M. persicae*, *M. ascalonicus* and *M. caryella* (Table 1). We also analyzed the body contents of three other whitefly species: *T. vaporariorum*, *T. abutilonea* and *T. acaciae*, for the presence of sorbitol. Mannitol, but not sorbitol, was present in the body extracts of all four aphid species examined. In every instance, the polyol content of each species of insects collected at noon was 8–20-fold higher than in those collected from the same plants during the early morning (data not shown).

Crude aqueous extracts of *A. gossypii* were analyzed for the presence of reductase/dehydrogenases which might synthesize mannitol or one of its possible precursors. These extracts were unable to catalyze reduction of Fru-6-P, Man-1-P or Man-6-P with either NADPH or NADH (data not shown). Similarly, no reductase activity was detected in *A. gossypii* extracts when glucose was used as the hexose substrate (Table 2). However, aphid extracts catalyzed the reduction of fructose, exhibiting much higher rates of fructose reduction with NADPH than with NADH.

The ability of *A. gossypii* extracts to reduce fructose in the presence of NADPH indicates that mannitol synthesis in aphids may be catalyzed by a NADPH-requiring mannitol dehydrogenase (D-mannitol: NADP⁺ 2-oxidoreductase). To determine if the product of the NADPH-dependent fructose reduction was indeed mannitol, the products of aphid extract enzyme assays were analyzed by HPLC. For comparison, the products of whitefly extracts incubated with fructose and either

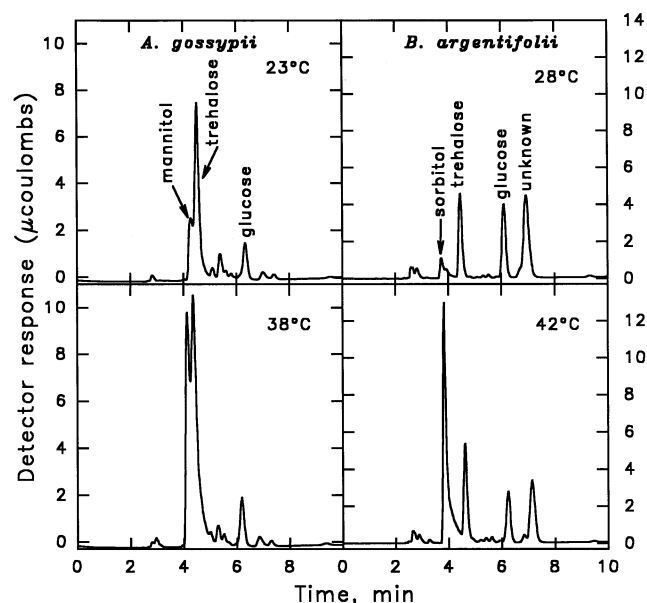


Fig. 3. Polyols and sugars in extracts of *A. gossypii* and *B. argentifolii* collected from cotton plants at various temperatures. Details of polyol and sugar analyses as for Fig. 1.

Table 1
Occurrence of mannitol and sorbitol in aphid and whitefly species

Insect species	Plant host ^a	Mannitol	Sorbitol
Aphids			
<i>A. gossypii</i> Glover	<i>G. hirsutum</i> L.	+	—
<i>M. persicae</i> Sulzer	<i>G. hirsutum</i> L.	+	—
<i>M. ascalonicus</i> Doncaster	<i>B. oleracea</i> var. <i>acephala</i> DC	+	—
<i>Monelia caryella</i> Fitch	<i>C. pecan</i> Engler and Graebn.	+	—
Whiteflies			
<i>B. argentifolii</i> Bellows and Perring	<i>G. hirsutum</i> L.	—	+
<i>T. abutilonea</i> Haldeman	<i>G. hirsutum</i> L.	—	+
<i>T. vaporariorum</i> Westwood	<i>L. esculentum</i> Mill.	—	+
<i>T. acaciae</i> Quaintance	<i>B. variegata</i> L.	—	+

^a Plant from which collected.

NADPH or NADH were also analyzed by HPLC. The resulting chromatograms of these reaction mixtures showed that aphid extracts converted fructose to mannitol in the presence of NADPH. *A. gossypii* extracts also carried out this reaction with NADH (Fig. 4) but at a considerably slower rate than with NADPH (Table 2). Sorbitol and mannitol were both synthesized from extracts of *A. gossypii* incubated with fructose and NADH (Fig. 4); however, the preferred reaction in vivo appears to be the creation of mannitol, since sorbitol was not detected in extracts of aphid bodies (Figs. 1 and 3). Extracts from *B. argentifolii* catalyzed the conversion of fructose only to sorbitol when incubated with either NADPH or NADH (Fig. 4).

Since *A. gossypii* extracts produced a small amount of sorbitol when incubated with fructose and NADPH (Fig. 4), one might expect that the polyol-forming enzymes in aphids and whiteflies might be similar. However, antibodies directed against the unusual NADP(H)-KR/SDH purified from *B. argentifolii* [26] failed to detect the presence of a cross-reacting protein in extracts of the cotton aphid (Fig. 5). These antibodies did recognize an immunoreactive polypeptide in extracts prepared from the silverleaf whitefly, *B. argentifolii*, and a polypeptide in extracts from the greenhouse whitefly, *T. vaporariorum* (Fig. 5).

Table 2
Hexose reductase activities in the cotton aphid and the silverleaf whitefly

Substrate	Coenzyme	Activity (IU ^a mg protein ⁻¹)	
		<i>A. gossypii</i>	<i>B. argentifolii</i>
Glucose	NADPH	0 ^b	0
Fructose	NADPH	0.61 ± 0.04	1.06 ± 0.07
Glucose	NADH	0	0
Fructose	NADH	0.19 ± 0.01	0.12 ± 0.02

^a IU, $\mu\text{mol min}^{-1}$.

^b Activity not detected.

Honeydew from *B. argentifolii* and *A. gossypii* consisted of a complex mixture of sugars (Fig. 6). Sugars in these excretions were considerably different from those in body extracts. Very little sorbitol or mannitol were found in whitefly or aphid honeydew, even in honeydew collected from insects feeding at elevated temperatures.

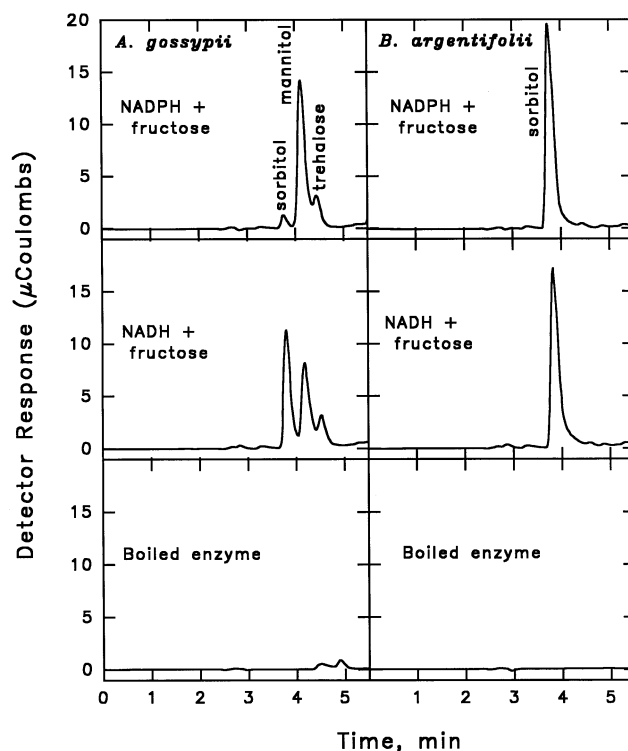


Fig. 4. The polyol products of ketose reduction by extracts of *A. gossypii* and *B. argentifolii*. Anion-exchange HPLC profiles of reaction mixtures prepared by incubating soluble extracts of *A. gossypii* or *B. argentifolii* for 3 h with fructose and either NADPH (top panels) or NADH (middle panels). The bottom panels show HPLC profiles of reaction mixtures that contained fructose, NADPH and extracts of *A. gossypii* and *B. argentifolii* in which proteins had been denatured by heating in a boiling water bath prior to incubation with fructose and NADPH.

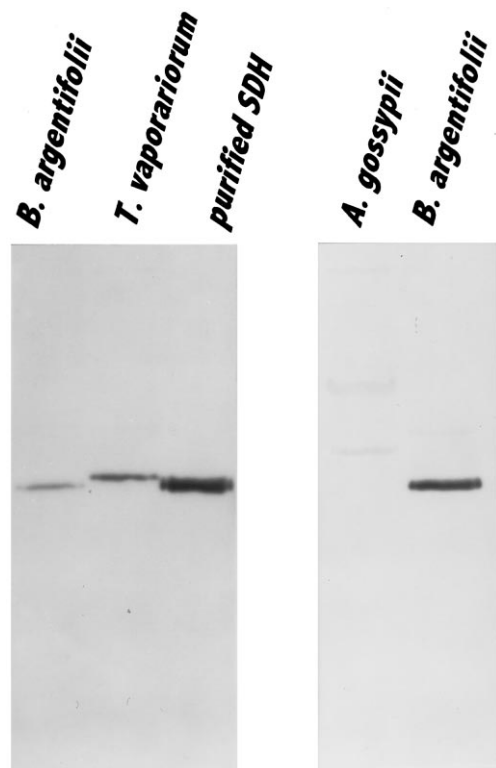


Fig. 5. Western blot analysis of NADPH-dependent KR/SDH in two whiteflies and an aphid. Polypeptides in soluble extracts from *B. argentifolii*, *T. vaporariorum* and *A. gossypii* were separated by SDS-PAGE, transferred to PVDF membrane and probed with polyclonal antibodies directed against NADPH-dependent KR/SDH from *B. argentifolii*. Purified NADPH-dependent KR/SDH from whiteflies was included as a standard.

4. Discussion

The accumulation of mannitol in aphids appears to be analogous to sorbitol accumulation in whiteflies. As in whiteflies, the polyol content of aphid bodies was much higher in early afternoon than in early morning. High air temperatures stimulated polyol accumulation in both aphids and whiteflies. Measurements of enzyme activity and product/precursor studies showed that both groups of insects used fructose as the substrate and NADPH as the coenzyme for polyol synthesis. However, mannitol was the major product of ketose reduction in aphids, while sorbitol was the only polyol product detected in whitefly extracts.

In many cold-hardy insects and in mammals, sorbitol is an intermediate in the conversion of glucose and fructose. In mammals, sorbitol is synthesized from glucose by a NADPH-dependent aldose reductase [2,13,18,27,28]. The catabolism of sorbitol to fructose is catalyzed by SDH, an obligate NADH-requiring enzyme [35] which is localized in mammalian liver, eye lens and reproductive tissues [18,28]. Both NADH- and NADPH-specific SDHs have been reported in insects

[5,32]. In some cold-hardy insects, such as *Bombyx mori* L., and *Eurosta solidaginis* Fitch, sorbitol accumulates at the beginning of diapause from glycogen-derived sugars [8,31,32]. In *E. solidaginis*, the trigger for sorbitol synthesis is exposure to temperature near 0°C [31–33]. As in mammals, the conversion of glucose to sorbitol in these cold-tolerant insects is carried out by a NADPH-requiring aldose reductase. At the end of diapause, an NAD⁺-specific SDH in *B. mori* and *E. solidaginis* converts sorbitol to fructose which is eventually reconverted to glycogen.

We have found no evidence in whiteflies for the pathway of sorbitol metabolism that is typical of cold-resistant insects [3,8,20,24,30,32,33]. Instead, synthesis of sorbitol in whiteflies involves reduction of fructose by a unique KR/SDH enzyme which is quite similar in structure to the catabolic NAD⁺-SDH in mammals (Wolfe et al., unpublished) but uses NADP(H) as a coenzyme [26,38]. Unlike the sorbitol-producing cold-tolerant insects discussed previously, we found no evidence that sorbitol is converted to (or is manufactured from) glucose in whiteflies.

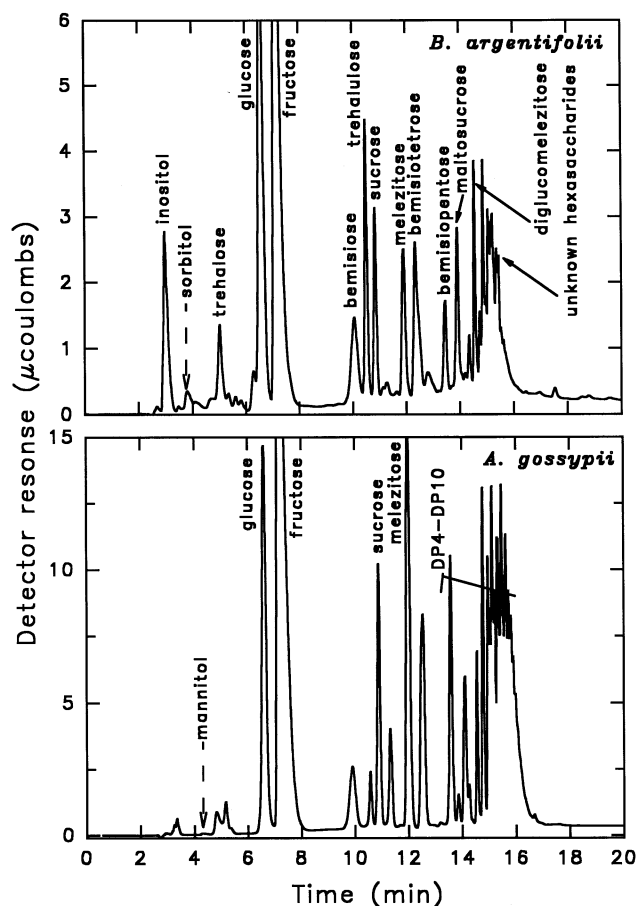


Fig. 6. Anion-exchange HPLC profiles of the honeydew from *A. gossypii* and *B. argentifolii*. Dashed-double dotted arrow in upper panel indicates the elution time of sorbitol. Dashed-double dotted arrow in lower panel indicates the elution position of mannitol.

The fact that *A. gossypii* extracts catalyzed sorbitol synthesis from NADH but not NADPH indicates that aphids contain the normal catabolic SDH but not the anabolic NADP(H)-KR/SDH found in whiteflies. Results of Western blot analysis using antibodies to the whitefly NADP(H)-KR/SDH support this conclusion.

As phloem-feeding insects, aphids and whiteflies ingest a diet extraordinarily rich in sugars. In many plant species, including cotton, sugars in the phloem sap upon which these insects feed consists almost exclusively of sucrose [34]. The concentration of sucrose in plant phloem sap varies diurnally [23] and can range from 200 to 600 mM [12]. These insects readily convert dietary sucrose to glucose and fructose by means of sucrase [25], which in aphids is localized in gut tissue [1]. In higher animals, glucose produced from sucrose by this enzyme can be readily incorporated into many biological reactions. However, the metabolic paths available for the fructose released by sucrase are far more limited. The conversion of fructose to polyols may therefore have arisen in part as a mechanism of dealing with high levels of dietary fructose which would be toxic if large quantities of this reducing sugar were absorbed by insect cells [3]. Interestingly, there was very little fructose in body extracts of whiteflies and aphids, even when polyol levels were low. Presumably, under conditions where polyols do not accumulate, fructose within the insects' bodies is either converted to glucose or excreted in honeydew. Sorbitol and mannitol are never more than trace components in the honeydew secreted by these insects.

The honeydews secreted by different insect species vary considerably both in the sugars they contain and in the relative abundance of these sugars. For instance, even fairly closely related whitefly species excrete honeydews with distinctively different sugar compositions [9,16,17]. Different species of aphids also secrete honeydews with distinctive sugar compositions. For example, most aphids excrete large amounts of melezitose [*O*- α -D-glucopyranosyl-(1 \rightarrow 3)-*O*- β -D-fructofuranosyl-(2 \leftrightarrow 1)- α -D-glucopyranoside] in their honeydew [16], but we failed to detect this trisaccharide in extracts from the aphid bodies we analyzed. Sugars larger than trisaccharides are commonly found in both aphid [11] and whitefly [9,16,36,37] honeydews but they were not observed in extracts of the bodies of these insects. In spite of the considerable variation in their honeydew sugar compositions, all four aphid species we examined accumulated mannitol and all four of the whitefly species accumulated sorbitol.

The six carbon polyols do not readily cross membranes, which is one of the major reasons for their value as osmoprotectants in animal systems [13,35]. The levels of sorbitol in whiteflies and mannitol in aphids exhibited pronounced diurnal fluctuations. Since they

do not appear to be excreted, the diurnal decrease we observed in the insect's sorbitol or mannitol content seems to be the result of the metabolic conversion of these polyols to other compounds. It would seem reasonable to conclude that in both aphids and whiteflies these polyols are synthesized in the hemolymph, where they remain until reconverted to fructose.

In terms of insect survival, polyols have long been recognized as providing protection against freezing stress. The insects analyzed in this study are not frequently subject to freezing, but they do experience osmotic stress from their diet of concentrated sugar solution and both thermal and water stress in their arid environment. In a previous study we presented evidence that sorbitol accumulation in *B. argentifolii* provides a mechanism for thermo- and osmoprotection [38]. Evidence presented here suggests that mannitol functions in a similar capacity in aphids.

Acknowledgements

We express our appreciation for the technical assistance provided by Terry Steele and Steve Waichulaitis. We are also grateful for samples of the greenhouse whitefly from Dr. Tom Perring, University of California, Riverside.

References

- [1] Auclair JL. Aphid feeding and nutrition. *Annu Rev Entomol* 1963;8:439–89.
- [2] Bagnasco SM, Uchida S, Balaban RS, Kador PF, Burg MB. Induction of aldose reductase and sorbitol in renal inner medullary cells by elevated extracellular NaCl. *Proc Natl Acad Sci USA* 1987;84:1718–20.
- [3] Becker A, Schlöder P, Steele JE, Wegener G. The regulation of trehalose metabolism in insects. *Experientia* 1996;52:433–9.
- [4] Bielecki RL. Sugar alcohols. In: Loewus FA, Tanner W, editors. *Encyclopedia of Plant Physiology*, N.S., vol. 13A. Berlin: Springer Verlag, 1982:158–92.
- [5] Bischoff WL. Genetic control of soluble NAD-dependent sorbitol dehydrogenase in *Drosophila melanogaster*. *Biochem Genet* 1976;14:1019–38.
- [6] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. *Anal Biochem*. 1976;72:248–59.
- [7] Brünker P, Altenbuchner J, Kulbe KD, Mattes R. Cloning, nucleotide sequence and expression of a mannitol dehydrogenase gene from *Pseudomonas fluorescens* DSM50106 in *Escherichia coli*. *Biochem Biophys Acta* 1997;1351:157–67.
- [8] Chino H. Enzymatic pathways in the formation of sorbitol and glycerol in the diapausing egg of the silkworm, *Bombyx mori*—I. On the polyol dehydrogenases. *J Insect Physiol* 1960;5:1–15.
- [9] Davis DW, McDougall EM, Hendrix DL, Steele TL, Adaskaveg J, Butler E. Air particulates associated with the ash whitefly. *J Air Waste Manag Assoc* 1993;43:1116–21.

- [10] Erarslan A. The effect of polyol compounds on the thermostability of penicillin G acylase from a mutant of *Escherichia coli* ATCC 11105. *Proc Biochem* 1995;30:133–9.
- [11] Fisher DB, Wright JP, Mittler TE. Osmoregulation by the aphid *Myzus persicae*: a physiological role for honeydew oligosaccharides. *J Insect Physiol* 1984;30:387–93.
- [12] Fisher DB, Gifford RM. Accumulation and conversion of sugars by developing wheat grains. VI. Gradients along the transport pathway from the peduncle to the endosperm cavity during grain filling. *Plant Physiol* 1986;82:1024–30.
- [13] Garcia-Perez A, Burg MB. Role of organic osmolytes in adaptation of renal cells to high osmolality. *J Membr Biol* 1991;119:1–13.
- [14] Gehrken U. Winter survival of an adult bark beetle *Ips acuminatus* Gyll. *J Insect Physiol* 1984;30:421–9.
- [15] Hendrix DL, Peelen KK. Artifacts in the analysis of plant tissues for soluble carbohydrates. *Crop Sci* 1987;27:710–5.
- [16] Hendrix DL, Wei Y-A, Leggett JE. Homopteran honeydew sugar composition is determined by both the insect and plant species. *Comp Biochem Physiol* 1992;101B:23–7.
- [17] Hendrix DL, Wei Y-A. Bemisiose: an unusual trisaccharide in *Bemisia* honeydew. *Carbohydr Res* 1994;253:329–34.
- [18] Iwata T, Carper D. Human sorbitol dehydrogenase gene. cDNA sequence and expression. In: Weiner H, Holmes RS, Wermuth B, editors. *Enzymology and Molecular Biology of Carbonyl Metabolism*, V. New York: Plenum Press, 1995:373–81.
- [19] Jeffery J, Jörnvall H. Sorbitol dehydrogenase. *Adv Enzymol* 1988;61:47–106.
- [20] Lee RE, Denlinger DL. *Insects at Low Temperature*. New York: Chapman and Hall, 1991.
- [21] Loos H, Krämer R, Sahn H, Sprenger GA. Sorbitol promotes growth of *Zymomonas mobilis* in environments with high concentrations of sugar: evidence for a physiological function of glucose–fructose oxidoreductase in osmoprotection. *J Bacteriol* 1994;176:7688–93.
- [22] Miller LK, Smith JS. Production of threitol and sorbitol by an adult insect: association with freezing tolerance. *Nature* 1975;258:519–20.
- [23] Mitchell DE, Gadus MV, Madore MA. Patterns of assimilate production and translocation in muskmelon (*Cucumis melo* L.). *Plant Physiol* 1992;99:959–65.
- [24] Niimi T, Yamashita O, Yaginuma T. A cold-inducible *Bombyx* gene encoding a protein similar to mammalian sorbitol dehydrogenase. Yolk nuclei-dependent gene expression in diapause eggs. *Eur J Biochem* 1993;213:1125–31.
- [25] Salvucci ME, Wolfe GR, Hendrix DL. Effect of sucrose concentration on carbohydrate metabolism in *Bemisia argentifolii*: biochemical mechanism and physiological role for trehalulose synthesis in the silverleaf whitefly. *J Insect Physiol* 1997;43:457–64.
- [26] Salvucci ME, Wolfe GR, Hendrix DL. Purification and properties of an unusual NADPH-dependent ketose reductase from the silverleaf whitefly. *Insect Biochem Molec Biol* (in press); 1998.
- [27] Smardo FL Jr, Burg MB, Garcia-Perez A. Kidney aldose reductase gene transcription is osmotically regulated. *Am J Physiol* 1992;262:776–82.
- [28] Smith MG. Polyol dehydrogenases. 4. Crystallization of the L(+)-iditol dehydrogenase of sheep liver. *Biochem J* 1962;83:135–44.
- [29] Sømme L. Mannitol and glycerol in overwintering aphid eggs. *Nor J Entomol* 1969;16:107–11.
- [30] Sømme L. Supercooling and winter survival in terrestrial arthropods. *Comp Biochem Physiol* 1982;73A:519–43.
- [31] Storey KB, Baust JG, Storey JM. Intermediary metabolism during low temperature acclimation in the overwintering gall fly larva, *Eurosta solidaginis*. *J Comp Physiol* 1981;144:183–90.
- [32] Storey KB, Storey JM. Biochemical strategies in overwintering in the gall fly larva, *Eurosta solidaginis*: effect of low temperature acclimation on the activities of enzymes of intermediary metabolism. *J Comp Physiol* 1981;144:191–9.
- [33] Storey JM, Storey KB. Regulation of cryoprotectant metabolism in the overwintering gall fly larva, *Eurosta solidaginis*: temperature control of glycerol and sorbitol synthesis. *J Comp Physiol* 1983;149:495–502.
- [34] Tarczynski MC, Byrne DN, Miller WB. High performance liquid chromatography analysis of carbohydrates of cotton-phloem sap and honeydew produced by *Bemisia tabaci* feeding upon cotton. *Plant Physiol* 1992;98:753–6.
- [35] Touster O, Shaw DRD. Biochemistry of the acyclic polyols. *Physiol Rev* 1962;42:181–225.
- [36] Wei Y-A, Hendrix DL, Nieman R. Isolation of a novel tetrasaccharide, bemisiotetrose, and glycine betaine from silver leaf whitefly honeydew. *J Agric Food Chem* 1996;44:3214–8.
- [37] Wei Y-A, Hendrix DL, Nieman R. Diglucomelezitose, a novel pentasaccharide in silverleaf whitefly honeydew. *J Agric Food Chem* 1997;45:3481–6.
- [38] Wolfe GR, Hendrix DL, Salvucci ME. A thermoprotective role for sorbitol in the silverleaf whitefly. *J Insect Physiol* (in press); 1998.